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## **Liver neoplasms in methylmalonic aciduria - an emerging complication**

Forny, Patrick ; Hochuli, Michel ; Rahman, Yusof ; Deheragoda, Maesha ; Weber, Achim ; Baruteau, Julien ; Grunewald, Stephanie

**Abstract:** Methylmalonic aciduria (MMA) is an inherited metabolic disease caused by methylmalonyl-CoA mutase deficiency. Early-onset disease usually presents with a neonatal acute metabolic acidosis, rapidly causing lethargy, coma and death if untreated. Late-onset patients have a better prognosis but develop common long-term complications, including neurological deterioration, chronic kidney disease, pancreatitis, optic neuropathy and chronic liver disease. Of note, oncogenesis has been reported anecdotally in organic acidurias. Here, we present three novel and two previously published cases of MMA patients who developed malignant liver neoplasms. All five patients were affected by a severe, early-onset form of isolated MMA (4 mut , 1 cblB subtype). Different types of liver neoplasms, i.e. hepatoblastoma and hepatocellular carcinoma, were diagnosed at ages ranging from infancy to adulthood. We discuss pathophysiological hypotheses involved in MMA-related oncogenesis such as mitochondrial dysfunction, impairment of tricarboxylic acid cycle, oxidative stress, and effects of oncometabolites. Based on the intriguing occurrence of liver abnormalities, including neoplasms, we recommend close biochemical and imaging monitoring of liver disease in routine follow-up of MMA patients. This article is protected by copyright. All rights reserved.

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## Title

Liver neoplasms in methylmalonic aciduria – an emerging complication

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## Abstract

Methylmalonic aciduria (MMA) is an inherited metabolic disease caused by methylmalonyl-CoA mutase deficiency. Early-onset disease usually presents with a neonatal acute metabolic acidosis, rapidly causing lethargy, coma and death if untreated. Late-onset patients have a better prognosis but develop common long-term complications, including neurological deterioration, chronic kidney disease, pancreatitis, optic neuropathy and chronic liver disease. Of note, oncogenesis has been reported anecdotally in organic acidurias. Here, we present three novel and two previously published cases of MMA patients who developed malignant liver neoplasms. All five patients were affected by a severe, early-onset form of isolated MMA (4 *mut*<sup>0</sup>, 1 *cblB* subtype). Different types of liver neoplasms, *i.e.* hepatoblastoma and hepatocellular carcinoma, were diagnosed at ages ranging from infancy to adulthood. We discuss pathophysiological hypotheses involved in MMA-related oncogenesis such as mitochondrial dysfunction, impairment of tricarboxylic acid cycle, oxidative stress, and effects of oncometabolites. Based on the intriguing occurrence of liver abnormalities,



including neoplasms, we recommend close biochemical and imaging monitoring of liver disease in routine follow-up of MMA patients.

### **Author contributions**

P.F. designed the study together with J.B. and S.G. Patient vignettes for cases 1 and 3 were contributed by M.H. and Y.R. Histological studies were performed by A.W. and M.D. The manuscript was written by P.F. together with J.B. and S.G. The guarantor of the study is S.G.

### **Compliance with ethics guidelines**

#### **Informed consent**

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. Anonymised data were collected retrospectively. Sample analysis of patient 2 was approved by the National Research Ethics Service Committee London - Bloomsbury (13/LO/0168). Written consent of patient, parents or legal carer was obtained for sample analysis (patients 2 and 3).

#### **Conflict of interest**

The authors declare no conflict of interest.

#### **Animal rights**

This article does not contain any studies with animal subjects performed by any of the authors.

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### **Take home message**

Liver neoplasms are a complication in MMA and warrant regular monitoring.

### **Key words**

Methylmalonic aciduria; liver, hepatoblastoma; hepatocellular carcinoma; mitochondrial dysfunction; oxidative stress; oncogenesis

## Introduction

Isolated methylmalonic aciduria (MMA) is an autosomal recessive disorder of propionate metabolism caused by mutations in the *MMUT* gene (*mut* subtype, OMIM: 251000) ([Forny et al 2016](#)) encoding methylmalonyl-CoA mutase (MUT, EC 5.4.99.2), which requires adenosylcobalamin as a cofactor. Failure to produce and deliver the cofactor to its target enzyme MMUT also results in MMA, involving mutations in the *MMAA* (*cblA* subtype, OMIM: 251100) and *MMAB* (*cblB* subtype, OMIM: 251110) genes.

Patients either present an early-onset disease with acute neonatal decompensation, associated with lethargy, vomiting, hypotonia, metabolic acidosis and hyperammonaemia, or a late-onset with symptoms such as failure to thrive, anorexia, vomiting and developmental delay. Patients, even when treated early, are at risk of long-term complications ([Horster et al 2007](#)), *i.e.* acute or chronic basal ganglia injury, white matter disease, optic neuropathy, tubulointerstitial nephritis leading to progressive renal failure, cardiomyopathy and pancreatitis. Recent guidelines have defined management of MMA patients, including monitoring and treatment of those complications ([Baumgartner et al 2014](#)).

MMUT has an anaplerotic role in supplying succinyl-CoA to the tricarboxylic acid cycle and its expression is particularly high in the liver. Liver-transplanted MMA patients present a reduction of metabolic decompensations and lower plasma levels of intermediary metabolites inherent to the disease. Despite the significant role of the liver in MMA metabolism, hepatic complications have been scarcely described. A recent study reported on longitudinal elevations of alpha-fetoprotein, the occurrence of hyperechoic liver tissue on ultrasound, and

marked pathological changes on liver biopsy, ranging from fibrosis to cirrhosis ([Imbard et al 2018](#)).

Here we present three unreported and two previously published cases of MMA patients ([Cosson et al 2008](#); [Chan et al 2015](#)) who developed liver neoplasms (hepatoblastoma and/or hepatocellular carcinoma). We discuss possible pathogenic mechanisms leading to oncogenesis in MMA and provide recommendations on monitoring liver complications in MMA patients.

## Patients and results

We present the detailed medical history for patients 1-3 and a summary of new and previously published cases (Table 1).

### Case 1

Patient 1 was born at term from non-consanguineous Caucasian parents. She presented at the age of 10 days with collapse and metabolic acidosis, requiring resuscitation, but diagnostic investigations were not conclusive. Subsequently she was noted to have motor developmental delay and presented again with vomiting and poor feeding at the age of 9 months, when the diagnosis of MMA was confirmed, showing compound heterozygous mutations in the *MMAB* gene (c.556C>T, c.643A>G). Conventional treatment was initiated, resulting in metabolic control, but she developed stage 4 chronic kidney disease. At 16 years of age, she required haemodialysis. Subsequently, she became more unstable and had about 3-4 admissions per year for acute metabolic decompensations, one of which was complicated by a basal ganglia

stroke, resulting in dysarthria and severe locomotor disability while her cognitive function was mainly spared. Ongoing nausea and occasional vomiting required a jejunostomy insertion to support nutrition. Her osteopenia (Z score of -5.7 at the spine, -5 at the total hip site, aged 19 years) was treated with bisphosphonates. At 22 years of age, a severe metabolic decompensation led to significantly raised lactate and mild hyperammonaemia. Despite intensive clinical management, she deteriorated and passed away a few days later. Concomitantly a liver ultrasound had shown a small lesion in the liver. A post-mortem report confirmed hepatocellular carcinoma on a background of cirrhosis and steatosis, which could have contributed to her poor acute treatment response.

## Case 2

Patient 2 is the younger brother of an affected sibling sharing the diagnosis of *mut*<sup>0</sup> MMA born to consanguineous parents. The index patient was diagnosed after neonatal presentation, had minor metabolic decompensations, but developed learning difficulties and autistic spectrum disorder. Parents opted out of prenatal genetic testing for patient 2, hence he was managed prospectively from birth. On antenatal scan he had been diagnosed with a right multicystic and dysplastic kidney, confirmed on postnatal ultrasound (Supp. Fig. 1AB) and magnetic resonance imaging (Supp. Fig. 1C), in addition depicting bilateral hydroureteronephrosis (Supp. Fig. 1CD). Postnatally, the diagnosis of MMA was confirmed (homozygous *MMUT* c.692dup) and the patient was started on conventional treatment. Despite metabolic stability, chronic mild hyperammonaemia around 150 µmol/L (Supp. Fig. 2A) required long-term ammonia scavengers. During routine monitoring at four months of age, elevated liver enzymes (gamma-glutamyl transferase and alkaline phosphatase) (Supp.

Fig. 2B) triggered detailed liver investigations, which revealed a heterogeneous, hyperechoic lesion (Fig. 1AB), as a mass in segment VII (Fig. 1C). Alpha-fetoprotein levels were peaking at a maximum of 23,780 ng/mL (reference <10) (Supp. Fig. 2C) and hepatoblastoma was detected in a liver biopsy. After multidisciplinary assessment, liver transplant was performed at six months of age, serving the dual purpose of removal of the tumour as well as supportive treatment of the underlying MMA. He received two subsequent courses of cisplatin to minimise the risk of metastases. Unexpectedly, the explanted liver showed foci of hepatocellular carcinoma in addition to hepatoblastoma. Screening for hepatitis B and C virus serotypes was negative. Intermittent post-transplant elevations of alanine and aspartate transaminases (Supp. Fig. 2D) were presumably caused by a viral infection, as transplant rejection and reoccurrence of tumour were excluded. Ten months post-transplant he remains relapse-free with no metabolic decompensations or complications from liver transplantation.

### Case 3

Patient 3 presented a few days after birth with generalized hypotonia, hypothermia, and dyspnoea. Increased methylmalonic acid in urine led to the diagnosis of MMA, confirmed by 1% of residual MMUT activity in cultured fibroblasts and compound heterozygous mutations in the *MMUT* gene (c.410C>T, c.655A>T). On a low-protein diet her metabolic control was satisfactory with 1-2 hospital admissions per year due to mild decompensations. She showed mild developmental delay, growth retardation and delayed puberty. She had low bone density (Z score of -2.0 at the spine, -2.1 at the total hip site, aged 30 years) and was diagnosed with Scheuermann's disease as teenager. Around the same age she developed chronic kidney disease stage 4. At the age of 23 years she developed bilateral visual loss due to optic atrophy,

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and bilateral partial neurosensory hearing loss. Concurrently, she presented two episodes of deep venous thrombosis. At the age of 31 years, baseline investigations during a mild metabolic decompensation showed a suspicious lesion on liver ultrasound, which was unapparent during routine ultrasound monitoring ten years prior. Magnetic resonance imaging confirmed one lesion in segment VI and a smaller lesion in segment V/VI, which on liver biopsy was diagnostic of hepatocellular carcinoma (negative screening for hepatitis B and C virus serotypes). Positron emission tomography computed tomography imaging did not reveal any metastases and liver segments V and VI were resected without perioperative complications. Histological investigations of the tumour confirmed a completely necrotic hepatocellular carcinoma with signs of fibrosis in the surrounding liver tissue. 17 months after tumour resection she remained relapse-free but passed away at the age of 32 years due to the sequelae of an acute haemorrhagic pancreatitis.

### **MMA severity and outcome**

All five patients included in the study (Table 1) presented during the first year of life without clinical hydroxocobalamin responsiveness. Common clinical findings included significant chronic kidney disease, high levels of plasma and urinary methylmalonic acid and markedly reduced MMUT activity in the cases investigated. Patients had comparable metabolic treatment with the mainstay of low protein diet, carnitine supplementation, and a glucose polymer-based emergency regime. The phenotypic severity in the presented cases is further underlined by the poor survival with three out of five patients having passed away between eleven (case 4) and 32 years (case 3) of age. In patients 1 and 4 the cause of death was partly attributed to the liver neoplasm.

### Genotype-phenotype correlation

All mutations of cases 1-4 were previously associated with a severe phenotype, except for the novel *MMAB* mutation c.643A>G p.(Arg215Gly) in case 1. Case 2 was homozygous for a truncating mutation resulting in p.(Tyr231\*), yielding no functional enzyme (Forny et al 2016). The common severe catalytic mutant p.(Asn219Tyr) (Forny et al 2014) was found in cases 3 and 4. Case 3 also harboured the p.(Ala137Val) mutant, a *mut*<sup>0</sup> allele in exon 3, which corresponds in a large part to the essential substrate-binding site of MMUT (Froese et al 2010), whereas case 4 carried the severe catalytic and folding mutant p.(Ala191Glu) (Forny et al 2014) in a compound heterozygous state. Case 1, the only non-*MMUT* case, carried the common p.(Arg186Trp) *MMAB* mutant (Lerner-Ellis et al 2006) alongside the novel p.(Arg215Gly) mutant, affecting residue 215, which is directly involved in the formation of the active site. All mutations found are non-responsive to hydroxocobalamin treatment *in vivo* or supplementation *in vitro*, further emphasising the severity of the cases presented in this study. For case 5, no mutational information was available.

### Histological findings

Microscopic studies revealed features of hepatoblastoma (cases 2, 4, 5) and hepatocellular carcinoma (cases 1-4); cases 2 and 3 were studied in more detail. The explanted liver of case 2 displayed mixed hepatoblastoma (Fig. 2A) with mesenchymal aspects (Fig. 2B). Remarkably, hepatocellular carcinoma elements were also present, featuring focal cytoplasmic beta catenin expression, expression of glutamine synthetase, glypican3 (not shown) and canalicular expression of bile salt export pump (Fig. 2C) without evidence for congenital hepatic fibrosis. The liver biopsy of case 3 showed vast areas of necrosis and other



hepatocellular carcinoma-typical elements, such as hepatocytic differentiation, loss of reticulin, and glutamine synthetase staining (Fig. 3A), indicative of carcinogenic WNT signalling, in line with detection of nuclear beta catenin (Fig. 3B). Upon liver resection, inflammation and necrosis were detected (Fig. 3C). Investigation of the lesion-surrounding tissue revealed portal and septal fibrosis and hepatocytes with glycogenated nuclei in cases 2 and 3 (Fig. 2D, Fig. 3D).

## Discussion

### Clinical presentation and histological findings

We describe three unreported cases of liver neoplasm associated with severe MMA presentation and reviewed two previously published patients. The five patient cohort carried mutations previously associated with severe phenotypes, presented an early-onset disease and renal, pancreatic and neurological complications. Liver neoplasms presented at ages ranging from 10 weeks (case 2, hepatoblastoma with areas of hepatocellular carcinoma) to 31 years (case 3, hepatocellular carcinoma) – both exceptionally early occurrences for these tumour entities. The concomitant finding of two different tumour entities (case 2) is intriguing as the mechanism of their emergence is fundamentally different. Hepatoblastoma and hepatocellular carcinoma develop by malignant transformation of foetal and well-differentiated hepatocytes, respectively. Cases 1-3 showed evidence of cirrhosis/fibrosis, as previously reported in MMA ([Imbard et al 2018](#)) and might *per se* increase the risk of developing liver neoplasms. Cirrhosis is a recognised complication in other inborn errors of metabolisms, such as Wilson disease, tyrosinaemia type I, argininosuccinic aciduria or glycogen storage disorders; the latter

three diseases identified with significant risk of developing hepatocellular carcinoma ([Schady et al 2015](#); [Baruteau et al 2017](#); [van Ginkel et al 2017](#)).

### **Toxic metabolites and mitochondrial dysfunction**

Mitochondrial dysfunction is a well-recognised pathophysiological mechanism in MMA: Megamitochondria, decreased mitochondrial mass, and impaired mitochondrial membrane potential in an animal model ([Chandler et al 2009](#)) and abnormal mitochondrial ultrastructure in patients ([Wilnai et al 2014](#)) have been reported. Increased fibroblast growth factor 21, a biomarker for mitochondrial disease, correlates with long-term complications in MMA ([Manoli et al 2018](#)). The mitochondrial pathophysiology is multifactorial (Fig. 4) ([Kolker et al 2013](#)): i) Accumulating propionyl-CoA inhibits pyruvate dehydrogenase complex ([Gregersen 1981](#)), succinate-CoA ligase, a key enzyme in producing and maintaining mitochondrial DNA, and the respiratory chain by a direct mechanism ([Schwab et al 2006](#)); ii) anaplerosis of the tricarboxylic acid cycle is impaired due to reduced succinyl-CoA production from defective MUT, causing a reduced tricarboxylic acid cycle flux to produce energy in mitochondria; iii) excessive 2-methylcitrate, produced from accumulating propionyl-CoA reacting with oxaloacetate, is a potent toxic metabolite, inhibiting various enzymes of the tricarboxylic acid cycle ([Cheema-Dhadli et al 1975](#)).

Subsequently to mitochondrial impairment, increased production of reactive oxygen species is suspected to play a major role in numerous MMA complications, such as optic neuropathy ([Pinar-Sueiro et al 2010](#)), chronic renal failure ([Manoli et al 2013](#)), and chronic liver disease ([de Keyzer et al 2009](#)). Similarly, increased oxidative stress is likely to be involved in liver oncogenesis, causing DNA damage and activation of reactive oxygen species-dependent pro-

oncogenic signalling pathways, including autophagy ([Azad et al 2009](#)), nuclear factor  $\kappa$ -B signalling ([Morgan and Liu 2011](#)), hypoxia-inducible factor 1- $\alpha$ , mitogen-activated protein kinase/ERK cascade, and the phosphoinositide-3-kinase/AKT pathway ([Kumari et al 2018](#)).

### **Impact of oncometabolites**

While toxic metabolites cause chronic tissue damage, independently posing a cancer risk, oncometabolites inflict neoplastic vulnerability via their effect on key-enzymes regulating metabolic pathways facilitating cell survival or dedifferentiation, mimicking the effect of mutations in tumour suppressor genes or oncogenes ([Erez and DeBerardinis 2015](#)). MMA oncometabolites may alter genome expression, *e.g.* propionyl-CoA is known to modify histone acetylation ([Nguyen et al 2007](#)). So far, three oncometabolites have been identified in organic acidurias: fumarate (fumarate hydratase deficiency), succinate (succinate dehydrogenase deficiency) and D-2-hydroxyglutarate (D-2-hydroxyglutaric aciduria type I and II). While evidence of oncometabolites in MMA is lacking, renal cell carcinoma kidneys of an MMA patient were found to carry a somatic knock-out mutation for the *TSC1* gene encoding hamartin ([Potter et al 2017](#)), shown to cause accumulation of fumarate ([Drusian et al 2018](#)). Hence, further genomic investigations of case 2 might help to understand their presentation of a multicystic dysplastic kidney. In addition, environmental factors such as exposure to exogenous hepatotoxic compounds or oncogenic viruses are parameters that need to be carefully taken into consideration in discussing the causative pathophysiological mechanisms of liver oncogenicity.

With regards to the liver, oncogenic processes might already be relevant before birth: Although the foetus benefits from maternal detoxification of toxic MMA metabolites *in utero*, preventing any systemic decompensation, the development of hepatoblastoma might be facilitated by the increased production of MMA-derived oncometabolites *in situ*, promoting oncogenicity in highly-proliferating foetal hepatocytes. Conversely, the development of hepatocellular carcinoma requires the transformation of mature hepatocytes, *e.g.* case 3. MMA might be another suitable disease model for the study of oncometabolites in inborn errors of metabolism.

### **Recommendations for monitoring of liver disease in MMA**

Approximately 50% of MMA patients show liver abnormalities ([Imbard et al 2018](#)). Liver monitoring, which involves a combination of yearly (biannually during the first year of life) liver enzymes (ALT, AST, ALP, GGT), alpha fetoprotein, and detailed liver ultrasound is crucial in MMA to detect chronic liver disease and neoplasm, especially in early-onset patients, who are unresponsive to hydroxocobalamin treatment. Immunosuppression, *e.g.* as required after kidney transplant (see case 4), is an additional risk factor for malignant transformation, warranting distinct attention ([Cosson et al 2008](#)). A transplanted liver does not foster the genetic defect but is still exposed to a – although lower – level of toxic metabolites, hence monitoring for liver neoplasms is equally necessary in these cases.

## Conclusion

With improved survival of MMA patients in the last decades, there is an increasing need to monitor these patients for long-term complications. Development of liver neoplasms in MMA might be an under-appreciated phenomenon. Although longitudinal and functional studies are required to better understand the pathophysiology, the occurrence of liver neoplasms in MMA might be multifactorial, cumulating multiple oncogenic events favoured by mitochondrial dysfunction, impairment of tricarboxylic acid cycle, oxidative stress, effects of toxic metabolites and potentially oncometabolites. Successful management of liver neoplasms requires early diagnosis and careful surveillance for liver neoplasms in the regular follow-up of MMA patients is recommended.

## Figures

### Figure 1

**Fig. 1. Liver imaging of case 2.** (A) A rounded, heterogeneous, predominantly hyperechoic approximately 2 x 2 cm lesion (B) with minimal internal vascularity was detected on ultrasound examination. (C) The lesion projects to segment VII of the liver (\*) and is relatively inconspicuous on computed tomography, which also depicts the multicystic dysplastic right kidney and pelvicalyceal/ureteric dilatation of both kidneys.

### Figure 2

**Fig. 2. Haematoxylin/eosin staining and immunostaining in liver histology of case 2.** (A) Explanted liver tissue displaying mixed hepatoblastoma comprising embryonal and foetal

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type epithelium; arrow indicates the embryonal component; inset shows nuclear beta catenin expression in the embryonal component (plus sign) and absent nuclear beta catenin expression in the adjacent foetal component (star). Bile salt export pump expression was not demonstrated in the hepatoblastoma component (not shown). **(B)** Mesenchymal elements were present in the form of osteoid. **(C)** A well differentiated hepatocellular carcinoma component demonstrating steatosis with unpaired arteriole-like vessels and stromal invasion; arrow in steatotic component indicates unpaired vessel expressing canalicular bile salt export pump (inset, small arrow). **(D)** The background liver demonstrated porto-portal fibrosis with mild steatosis, well glycogenated hepatocytes and mild porto-lobular activity.

### Figure 3

**Fig. 3. Histology of liver biopsy and resected liver of case 3.** **(A)** Biopsy from left to right displaying extensive necrosis and a tiny focus of vital cells, surrounded by inflammation. High power view revealing disturbed liver architecture and highly atypical cells with hepatocytic differentiation, silver stain (S) demonstrating focal loss of reticulin fibres, and immunostaining shows a strong reactivity for glutamine synthetase (GS) and **(B, BC)** nuclear beta catenin. **(C)** Liver resection showing entirely necrotic tumour nodules (overview), surrounded by a rim of fibrosis and inflammation (inset, plus sign) and shadowy necrotic tumour cells, reminiscent of hepatocellular carcinoma (inset, star). **(D, Sirius red stain on right)** Liver resection displaying subtle changes in the non-tumorous tissue including some portal tracts lacking clearly identifiable portal vein branches (stars), occasional foci of mostly portal inflammation (arrow heads), and occasional hepatocytes with glycogenated nuclei

(arrows). Stainings are haematoxylin/eosin except where specifically mentioned; immunostainings performed as previously described ([Friemel et al 2015](#)).

#### Figure 4

**Fig. 4. MMUT deficiency induces various metabolic disturbances promoting oncogenesis in MMA.** Orange arrows with circled minus indicate inhibitory effects; blue arrows indicate metabolic pathways; dashed arrows blue arrows indicate the reaction of accumulating propionyl-CoA with oxaloacetate to form 2-methylcitrate (2-MCA) and the accumulation of methylmalonic acid (MMAcid); black arrows indicate causal relationships supported by literature; the black dashed arrow suggests a potential origin of oncometabolites in MMA. MUT, methylmalonyl-CoA mutase; PDHc, pyruvate dehydrogenase complex; TCA cycle, tricarboxylic acid cycle; mtDNA, mitochondrial DNA; key metabolites in grey; small upwards arrows indicate increase of compounds.

#### Supplementary Figure 1

**Supp. Fig. 1. Ultrasound and magnetic resonance imaging of kidneys of case 2.** (A) Large right multicystic dysplastic kidney with (B) severe pelvicalyceal and ureteric dilatation, (C) as confirmed on magnetic resonance T2-weighted imaging with contrast. (D) The left kidney is normal in size however bright in echogenicity with moderate pelvicalyceal and ureteric dilatation.

#### Supplementary Figure 2

**Supp. Fig. 2. Long-term biochemical monitoring of case 2.** (A) Ammonia (NH<sub>3</sub>) levels (reference <40 µmol/L) improved after liver transplant (LT, dashed vertical line) at the age of

6 months. **(B)** Elevations of alkaline phosphatase (ALP, reference range 70-347 U/L) and gamma-glutamyl transferase (GGT, reference range 20-132 U/L) normalised after LT. **(C)** Significantly raised alpha-fetoprotein (AFP, reference <10 ng/mL) as one of the diagnostic markers of hepatoblastoma was no longer in the pathological range after tumour resection. **(D)** Alanine transaminase (ALT, reference range 9-40 U/L) and aspartate transaminase (AST, reference range 21-80 U/L) levels were interpreted to have peaked post-transplant due to a viral cause, affecting the newly implanted liver.



**Table 1**

	Case 1	Case 2	Case 3	Case 4	Case 5
<b>Demographics</b>					
Gender	Female	Male	Female	Male	Male
Ethnicity	Caucasian	Pakistani	Caucasian	Caucasian	N/A
<b>MMA</b>					
Age of diagnosis	10 days	5 days <sup>&amp;</sup>	5 days	10 days	on NBS
Onset <sup>β</sup>	Early	Early	Early	Early	Early
Clinical hydroxocobalamin responsiveness	No	No	No	No	No
Genotype	<i>MMAB</i> : c.556C>T p.(Arg186Trp), c.643A>G p.(Arg215Gly)	<i>MUT</i> : homozygous c.692dup p.(Tyr231*)	<i>MUT</i> : c.410C>T p.(Ala137Val), c.655A>T p.(Asn219Tyr)	<i>MUT</i> : c.572C>A p.(Ala191Glu), c.655A>T p.(Asn219Tyr)	N/A
MMUT activity (fibroblast studies)	N/A	N/A	1% of control	Undetectable	N/A
Plasma/urinary MMA level <sup>ω</sup>	plasma MMA (median): 1760 μmol/L (range 310 to 3300)	plasma MMA (median): 134 μmol/L (range 50 to 172)	plasma MMA (median): 1730 μmol/L (range 221 to 3420)	urinary MMA: 3.69 to 4.70 mmol/mmol creatinine	N/A

<b>Liver disease/neoplasm</b>					
Age at diagnosis of liver neoplasm	22 yrs 5 mos	4 mos 1 wk	30 yrs	11 yrs	1 yr 7 mos
Elevated liver enzymes	ALP, ALT	ALP, GGT	ALP, AST, GGT	ALT, AST	N/A
Alpha-fetoprotein levels (normal <10) [ng/mL]	N/A	23,780	9	73	500,000
Subtype of liver neoplasm	HCC	HB, HCC	HCC	HB (resembling adult HCC)	HB
Treatment of liver neoplasm	Neoplasm detected on post-mortem	Liver transplantation at age 6 months with subsequent chemotherapy (two cycles of cisplatin)	Resection of tumour	Neoplasm detected on post-mortem	Chemotherapy (six cycles of cisplatin, vincristine, 5-fluorouracil) and subsequent combined liver-kidney transplant
<b>Renal disease</b>					
Stage of renal disease	CKD 4	CKD 2	CKD 4	CKD 4	N/A
corrGFR [ml/min/1.73m <sup>2</sup> ]	22	87	24	20	N/A
Kidney transplant	No	No	No	Yes (at 9 yrs 8 mos of age)	Yes (at 2 yrs 3 mos of age as domino liver-

					kidney transplant)
<b>Outcome</b>					
Survival	Deceased (at 22 yrs 5 mos)	Alive	Deceased (at 32 yrs)	Deceased (at 11 yrs)	Alive
Cause of death	Acute metabolic decompensation with potential contribution of liver neoplasm	N/A	Acute hemorrhagic pancreatitis	Directly related to liver neoplasm	N/A

**Table 1. Overview of five MMA cases who presented with a liver neoplasm.** Information was extracted from literature for published cases 4 (Cosson et al 2008) and 5 (Chan et al 2015). NBS, newborn screening; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyltransferase; HB, hepatoblastoma; HCC, hepatocellular carcinoma; N/A, not available; CKD, chronic kidney disease; corrGFR, glomerular filtration rate, corrected for body surface.

<sup>&</sup> Diagnosis made upon sibling screen.

<sup>β</sup> Early corresponds to neonatal onset, *i.e.* ≤28 days of age.

<sup>ω</sup> Plasma MMA levels were assessed in metabolically well-controlled state and are based on 15 (case 1), ten (case 2, pre-transplant), and 14 (case 3) individual measurements, collected over a period of 2 years (case 1), 6 months (case 2), and 4 years (case 3), respectively.

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Fig. 1











